THE EFFECTS OF OCTACOSANOL ON CONCEPTION AND REPRODUCTION, ON MAINTENANCE AND GROWTH OF YOUNG, AND ON OXYGEN UPTAKE IN THE WHITE RAT

by

PATRICIA ROSE FARRELL

B. S., Mount Saint Scholastica College, 1963

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1966

Approved by:

Major Professor

TABLE OF CONTENTS

LITERATURE REVIEW

Octacosanol-1 is an octacosyl alcohol (CH_3-$(CH_2)_{26}$-CH_2OH) with a molecular weight of 410.75 and a melting point of 83.2-83.4 C. It can be isolated from a number of plant substances: wheat wax, carnauba wax, candelilla wax, wool wax, alfalfa wax, sugar cane cuticular wax, the leaves of Ginkgo biloba and Ephedra gerardina (Neufeld, 1963), the leaves of Sarcococca pruniformis (Gopinath, Kohli, and Kidwai, 1962), and the cotton plant (Sadykov, Isaw, and Ismailov, 1963).

For this study we are interested in octacosanol as a component of wheat wax. Wheat germ and wheat germ oil are known to contain factors which have varied and extensive effects. Octacosanol is probably one of the factors responsible for some of the observed effects.

Wheat germ oil may possess antioxidant properties. Tracy, Hoskisson, and Trimble (1944) demonstrated that wheat germ oil prevented rancidity when incorporated into certain dairy products at a level of approximately 0.2% of the weight of the fat content of the product. White, Handler, Smith and Stetten (1959) acknowledged the presence of a material in oil from wheat germ that inhibited the development of rancidity and oxidative destruction of vitamin A in natural fats. The active material was designated as vitamin E.

Wheat germ oil has caused favorable results when used in the treatment of muscular dystrophy and related dysfunctions. A substance in wheat germ oil, not necessarily vitamin E, passed from mother to pups in the milk acted to prevent paralysis which often developed one or two days before weaning in rats on a simplified diet. The paralysis involved severe degeneration of skeletal muscles. About 35% of the afflicted young died, 17% recovered from paralysis and 48% showed some permanent paralysis. The dosage level of

wheat germ oil adequate to prevent muscular degeneration was found to be below the level necessary for facilitation of the birth of live young (Evans and Burr, 1928; Goettsch and Ritzman, 1939). In some instances wheat germ oil was also effective in treatment of neuromuscular disturbances or disorders of the human nervous system. It was proposed that the preventive or curative agent in wheat germ oil was vitamin E and that it regulated tissue oxygen utilization since muscular dystrophy involved disturbances of muscle cell oxidation processes and a rise in oxygen consumption in the dystrophic muscle of about 250% above normal (Stone, 1949).

Unmodified wheat germ oil caused a decrease of creatinurea in patients with dermatomyositis. When the wheat germ was incubated in vitro with normal human gastric juice, the effect was decreased. When the wheat germ was defatted by extraction with ethylene dichloride the effect was eliminated altogether (Milhorat, Toscani, and Bartels, 1945).

Estrogenic, androgenic, and gonadotropic effects of wheat germ oil constituted an area of extensive study. Wheat germ oil produced by cold pressing had no estrogenic, gonadotropic, or luteinizing effects on immature, normal, or castrate female rats. Androgenic activity of wheat germ oil was demonstrated by an increase in seminal vesicle weight of castrate male rats, by an increase in comb size of full grown capons, and by an increase in comb weight of one to five day old chicks. A progestational effect was shown by endometrial proliferation in the rabbit, not observed in rabbits given alpha-tocopherol. Estrogenic, androgenic, and gonadotropic activities were attributed by Levin, Burns, and Collins to a ketonic compound (Neufeld, 1963). Topically applied natural or synthetic octacosanol caused a significant increase in comb growth of immature White Leghorn chicks (Levin, 1963).

Neufeld (1963) demonstrated a variation in comb growth response to octacosanol and wheat germ oil. In one experiment, octacosanol applied topically and wheat germ oil injected intraperitoneally produced significant results. In another experiment there was a significant increase in body weight with the highest level of octacosanol (0.22%), and lesser increases with lower levels. About the same increase as caused by wheat germ oil was obtained by the 0.11% level of octacosanol. White Leghorns showed significant results with all levels of wheat germ oil and octacosanol; indicating a strain specificity. Immature female mice fed wheat germ oil at the 5% level showed no estrogenic stimulation. At the 10% level there was an increase of uterine growth over the controls. Neither level of wheat germ oil produced any uterine growth in ovariectomized females.

Wheat germ and wheat germ oil have been repeatedly tested for effects on reproduction. Evans and Bishop (1923) were the first to recognize that wheat germ oil contained a factor essential for reproduction. They found that a diet of fat, carbohydrate, and protein with added minerals and vitamins A and the B complex maintained female rats as far as normal health and growth were concerned. The rats exhibited normal estrus, ovulation, fertilization, and implantation, but resorbed the embryos. Fresh green leaves, whole wheat, oats, and wheat embryo added to the diet corrected the sterility problem.

The factor was found in the unsaponifiable fraction of wheat germ oil and comprised about 0.3% of the oil (Evans and Burr, 1924); it was stable and could be stored by the body (Evans and Bishop, 1924). The newly isolated compound x was named vitamin "E" by Sure (1924a). The vitamin E complex was found to exist in ether extracts of yellow corn, wheat embryo, and hemp seed (Sure, 1924b).

Ether, acetone, and benzene extracts of wheat germ added to the diet of rats aided in the birth of healthy young and in lactation. Cottonseed oil, corn

oil, and palm oil supplied some of the factor but not to the same extent as wheat germ (Sure, 1926a). The possibility of two fat soluble dietary factors in wheat germ oil was suggested by Sure (1926b). Sure (1927) arranged vegetable oils, on the basis of their effect on fertility, in the following order of decreasing potency: wheat oil, crude corn, cottonseed oil, cocoa butter, and peanut oil which showed no potency.

Favorable results following the use of wheat germ oil as a treatment for sterility dysfunction of cows have been obtained (Asdell, 1949). The treatment was not considered to be synonomous with vitamin therapy as there were probably substances other than vitamin E in wheat germ oil that beneficially influenced reproduction (Gullickson et al., 1949; Marion, 1962).

Abortion has been prevented in humans by a large amount of wheat germ oil fed before conception and throughout pregnancy (Cromer, 1938; Watson and Tew, 1936).

Not all experiments with wheat germ oil resulted in such positive results. Only 60% of the cows treated with wheat germ oil for sterility conceived (Lentz, 1938). Wheat germ oil did not prevent or suppress Brucella abortus infection in cattle or guinea pigs (Gwatkin and Macleod, 1938). Failures of tests with wheat germ oil were reported by Card (1929), Titus and Burrows (1940), and Salisbury (1944).

Levin (1945) offered a possible explanation for the negative results obtained by certain workers using wheat germ oil and vitamin E. He speculated that the method of preparing the wheat germ oil and its subsequent treatment affected its chemical stability. He further suggested the possibility of the presence of nutritional factors other than vitamin E in wheat germ oil. The hypothesis was supported by the work of Levin, Sibernagel, and Nichols (Neufeld, 1963). They demonstrated that wheat germ oil in the diet of rats favorably

influenced conception, litter size, birth weight, and weaning weights. Both the control and experimental animals were on a diet supplemented with tocopherols so that vitamin E could not have been the factor of differentiation in that experiment.

Wheat germ oil was believed to contain a preventive or curative agent for growth deficiency. When wheat germ and wheat germ oil were added to a deficient diet for rats there was a resumption of growth. Some known vitamins added to the diet as supplements failed to bring about the resumption of normal growth. The curative agent in the wheat preparation was believed to be vitamin E (Blumberg, 1935).

Wheat germ oil was implicated as an influencing factor for growth of rats (Martin, 1937; Emerson and Evans, 1937). A factor necessary for normal growth of chicks was present in vegetable oils and fatty acid concentrates (Carver and Johnson, 1953). Wheat germ oil and oleic acid were especially functional in providing the factor for normal growth. The failure of alpha-tocopherol used alone to elicit a response seemed to rule out vitamin E as the curative factor.

Keane (1953) found that Sprague Dawley rats on a diet with linoleate as a fat source could rear only 2% or less of their young to weaning age. When the diet was supplemented with 2.5% wheat germ oil (VioBin), 86.6% of the young were reared successfully. Fat-free diets or diets supplemented with 5% lard did not increase the survival rate of the young. Corn oil and butter increased survival rates more than glyceryl trilaurate, lard, or fat-free diets. Keane concluded that the growth factor(s) in wheat germ oil and other natural fats such as corn oil and butter was not identical with the known vitamins or essential fatty acids. He attempted to devise a colorimetric assay for the factor by use of Fast Black Salt K (diazotized p-nitrobenzeneazodimethoxyanaline).

He believed his method measured the factor in question or a closely related one. He found the substance to be present primarily in the uterus complex and to a limited extent in the milk.

Another effect of wheat germ oil which had been widely studied was increase of stamina or working ability. The increase was believed to be brought about by an increase in the effeciency of the respiratory system. Guinea pigs on swimming tests were aided by wheat germ oil. All animals on natural rations drowned within 10 min. but 25-33% of the animals on corn oil ration swam for 60 min. at which time the tests were terminated. More than 60% of the animals fed a wheat germ oil ration were still swimming after 30 min. A ration containing 2% wheat germ oil was as effective as a ration containing 10% wheat germ oil. The addition of tocopherol to corn oil ration had no effect.

Comparable results were not obtained with rats fed wheat germ oil ration for 28 days preceding the tests. The rats on wheat germ oil ration did not swim longer than those on the normal ration (Ershoff and Levin, 1955). No significant difference was found in the time that rats could swim before tiring whether or not the rations were supplemented with octacosanol, wheat germ oil or vitamin E (Consolazio, et al., 1964).

Dietary supplements containing vitamin E and wheat germ oil had a positive influence in work performance tests for human subjects in strenuous physical training programs of long duration. Control subjects without the training program did not have their physical fitness test performances raised by the supplements. Improved relative metabolism, cardiovascular condition, and endurance performances of individuals fed wheat germ oil during training experiments supported the belief that wheat germ oil contained ergogenic aids other than vitamin E (Cureton, 1954).

Conclusions from a study of wheat germ oil as a dietary supplement in a program of conditioning exercises with middle-aged subjects were: (1) wheat germ oil was a valuable food supplement which helped the endurance of middle-aged men in running tread mill tests and in non-willpower tests; (2) wheat germ oil increased the speed of reaction of the whole body; (3) wheat germ oil tended to increase the oxygen uptake. The possibility of an individual sensitivity to wheat germ oil was indicated. Significant gains were shown for most subjects on wheat germ oil in all-out treadmill running and in tests of cardiovascular conditions (Cureton and Pohndorf, 1955).

Tuma (1959) stated that octacosanol was a substance which, when ingested in small quantities, increased physical endurance and aided heart response. His work also provided strong evidence that quantities above certain established minimums had a depressing effect. There was some evidence that phosphatides and other factors in oil aided in the absorption of octacosanol. The work provided evidence that octacosanol could be a part of the lipid co-enzyme of cytochrome reductase. Isolation of a compound from the lipo-protein of pig heart which had a configuration in which a long chain alcohol was a part was another result of the work.

No reports dealing directly with effects of wheat germ oil or octacosanol on oxygen uptake of rats were found although basic studies on respiration of rats have been reported. Quantitative assays on single animals have not been satisfactory because of statistical variations (MacLagan and Sheahan, 1950). A large number of animals must be used and results pooled to attain a reasonable degree of precision. The total variance on oxygen used in half hour periods with 17 rats was 154%; individual variance between rats was as high as 64% and variance between half hour periods within individual four hour trials was as high as 17%; resulting in a general random variance of 73% (Kleiber,

Smith, and Chernikoff, 1956).

By correlating activity with oxygen consumption, Davis and Hasting (1934) and Bramante (1959, 1961) partially explained the variability. Bramante plotted the volume of oxygen uptake and the activity of the rat against time. He found that with increased activity there was an increase in oxygen uptake which often lagged slightly. He determined the basal metabolic rate by choosing the oxygen uptake corresponding to the lowest activity. These measurements did not show as much variation as often reported with the use of other means of expression.

The individual variation so often observed could be due to a number of factors other than activity: sex, age, season, climatic conditions, species, type of diet, schedule of feeding, size and/or weight, cycles, and pregnancy (Zollner, 1962). Holtkamp et al. (1955) set some simple rules for limiting variations which commonly occur in measurement of oxygen uptake of rats: five animals should be used in each group; at least six animals, some from control and some from each treatment group, should be observed simultaneously under identical experimental conditions; the oxygen consumption rate of each animal should be analyzed individually.

In spite of the amount of work done with rat respiration, no definite patterns seem to have developed under which such experiments should be conducted or experimental results expressed. There are two main ways of measuring oxygen uptake--closed circuit methods and open circuit methods. Each has been widely used and has supporters, advantages, and disadvantages. Several types of apparatus have been developed for each method. In closed circuit methods, oxygen is added to the circuit and volume change recorded as the animal in the chamber utilizes the oxygen. Soda lime is commonly used as an absorbent for carbon dioxide.

Temperature of the animal chamber is noted and is often kept between 25 and 30 C. Measurements are best made after 12 to 21 hours of fasting. The activity of the animal is often controlled by the use of drugs. Sodium barbitone, sodium phenolbarbitone, Nembutal, Somnifaine, urethane, paraldehyde, chloroform, ether, and tritene are used (Davis and Hastings, 1934; Davis, 1938; Kleiber et al. 1956; Bramante, 1959; MacLagan and Sheahan, 1950).

The most commonly used means of expression of oxygen uptake were (1) metabolic rate per rat, (2) metabolic rate per surface area or per unit of the 2/3 power of body weight, (3) metabolic rate per unit weight, and (4) metabolic rate per unit of the 3/4 power of body weight (Kleiber, et al., 1956).

Activity or movement caused an increase in the oxygen uptake. After completion of work, ventilation and gaseous metabolism did not return immediately to the normal level. The increased oxygen uptake after cessation of the active performance of work was referred to as "oxygen debt" (von Noorden, 1907). Wachholder (1948) reported that after 30 minutes of work on a bicycle ergometer a human showed a gradual decrease of oxygen consumption through the debt period to a basal rate followed by fluctuations above and below the basal rate for hours following the actual work. The decrease below the basal rate increased with the absolute values of the basal rate of the subject and increased for the same subject with the severity of the work.

The purpose of this study was (1) to determine if excessive amounts of octacosanol would produce adverse effects, (2) to test whether these excessive amounts of octacosanol would produce any of the effects on reproduction attributed to wheat germ and wheat germ oil and (3) to determine specifically if any of several dosage levels of octacosanol would alter the amount of oxygen needed for recovery after exhausting exercise.

METHODS AND MATERIALS

Conception and Reproduction, Maintenance and Growth of Young

Three generations of Sprague Dawley rats were followed with records kept of matings, parturitions, number in litters on days 1, 5 and 21, weight of litters on day 21, and weights of selected organs of adult animals. The animals were designated by their identifying numbers with "C" for the controls and "E" for the experimentals; "0", "1" and "2" for the parental, first, and second generations; and "1", "2", "3", etc. for the individual animal of the group.

The parental generation of rats was obtained from Hormone Assay Laboratories, Chicago, Illinois. Both males and females were randomly divided into two groups which were maintained on their respective control and experimental diets. The female rats were kept in individual wire cages with wire rack bottoms. When 100 days old, they were placed with males for 15 days, the time corresponding to at least three estrous cycles. The females were returned to individual cages and 19 days after the day when male and female had been placed together, the wire racks were removed and wood shavings for nesting were placed in the tray. Wood shavings were kept in the cages until the pups were at least ten days old. The litter size was reduced to ten on the fifth day and litter separated from the dam and removed from the experiment on day 21. Members for the next generation were chosen from the second litters with special attention directed toward selecting the males and females from different litters. The old animals were then sacrificed. The entire procedure was repeated through a first generation and again through a second generation.

The rats were kept on a 16 hour light cycle and at a temperature of 80 ± 5 F. Water was available at all times. The rats were fed a basic diet

known to be adequate for normal health and reproduction (Table 1) with 1.0% NaCl added. The meal was mixed with water to form a mush and was dispensed into glass jars in quantities such that only a little feed remained along sides and bottoms of the jars after 24 hours. This was carefully watched especially during gestation and lactation when day to day records were kept on the amount of feed eaten by individual rats and their litters.

Table 1. Composition of the basic rat diet.

Ingredients	Amount/500 lbs.
Bulk[1].	
Soybean oil meal	50 lb
Ground yellow corn	61 lb
Alfalfa meal (17%)	15 lb
Edible animal fat	10 lb
Wheat middlings	69 lb
Premix A[1].	
Rolled oat groats	60 lb
Corn flakes	75 lb
Meat and bone scraps	75 lb
Fish meal	25 lb
Dried skim milk	20 lb
Dried brewers yeast	15 lb
Steamed bonemeal	5 lb
Salt	5 lb
Stapel	10 lb
Premix B[1].	
Vitamin D_3 (15,000 ICU/g)	50 g
Aurofac 10	681 g
Soybean oil meal	1439 g

[1]Items in Premix A and in Premix B were mixed then the two premixes blended with the "bulk" to effect a uniform mixture.

The experimental feed was prepared by adding 0.1% octacosanol (VioBin) on a weight to weight ratio of the basic feed and salt. For example: 6923 g of basic feed plus 70 g NaCl and 7 g octacosanol provided 7000 g of

0.1% experimental feed. The octacosanol was pulverized, then mixed with the salt and 100 g of feed. That mixture was added to the meal and the total thoroughly mixed in a large metal container.

After the adult rats had reared their second litters, they were euthanized with chloroform in a tightly closed jar. Total weight was taken. An incision was made on the midline on the ventral side. The kidneys, liver, spleen and heart were separated and weighed individually after removal of excess fat and blood.

Oxygen Uptake

Rats were randomly selected from the first and second litters of the animals maintained on the program previously described. The selected rats were maintained in the same manner. The 0.1% experimentals were continued on that diet. Groups for 0.01%, 0.001%, and 0.0001% levels and controls were chosen from litters of control animals. The high dilutions of octacosanol were prepared by one to nine mixtures of the previous level and stock feed.

Oxygen consumption was measured by the use of a "Minute Oxygen Uptake Spirometer" from Aloe Scientific (PLATE 1). The instrument measured extremely small volume changes in a closed system. It consisted of two main parts: the chamber which housed the experimental animals, and the compensating piston with the recording device. Some modifications were necessary to obtain satisfactory readings on the oxygen uptake of rats and mice. In Exp. 1, a pump with a capacity of 500 cc per minute was used to circulate the air from the chamber through a bottle containing a water absorbent (magnesium perchlorate[1] or sodium hydroxide) then another bottle containing a CO_2 absorbent (Mikohobite[1] or sodium calcium hydrate commonly called soda lime) then back to the chamber.

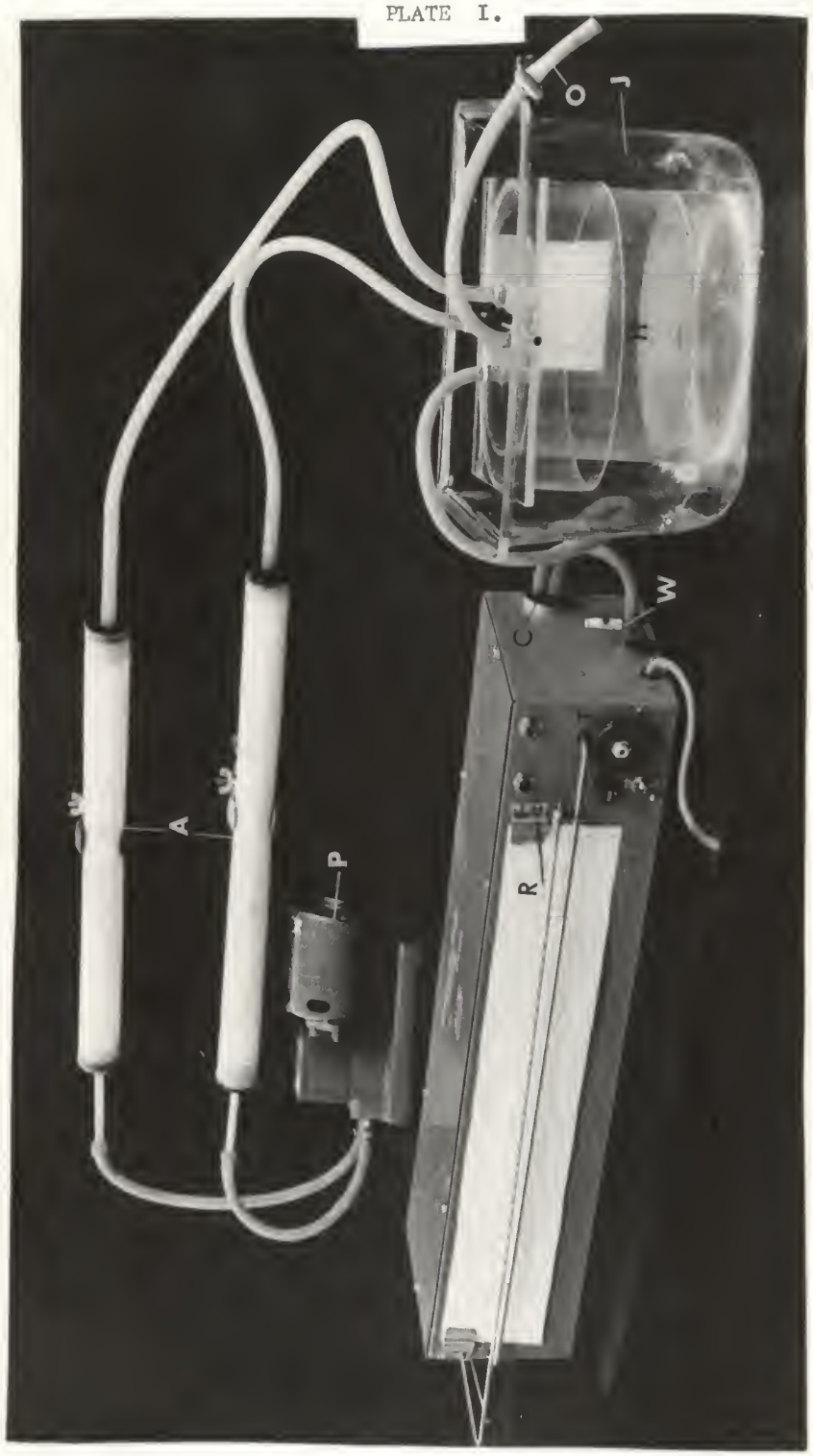
[1]Obtained from G. Frederick Smith Chemical Company, Columbus, Ohio.

EXPLANATION OF PLATE I

Minute oxygen uptake spirometer assembled for use except for attachment of the oxygen tank and flow of tap water through the jacket.

A. Tubes containing absorbents
   Upper--CaCl
   Lower--Soda Lime and CaCl
C. Cylinder and piston
H. Chamber
O. Oxygen source
P. Pump
R. Recording pen
J. Water jacket
T. Timer
W. Water level for compensating mechanism

PLATE I.



In Exp. 2 a pump with a capacity of 1500 cc per minute forced the air first through a plastic tube 2.8 x 35 cm containing $CaCl_2$, then through a second such tube with 25 cm soda lime and 9 cm $CaCl_2$, then back into the chamber. Ends of the tubes were plugged with glass wool to prevent movement of particles of the absorbent through the system. Repeated tests of the return air flow through a solution of barium hydroxide indicated near complete absorption of CO_2.

The chamber was closed by means of a water seal and circulation of tap water through the jacket kept the temperature within the chamber relatively constant. The chamber contained 3 liters of air. At the beginning of an experiment and two or three times during the experiment 300 cc oxygen was injected into the chamber to fill the cylinder, thus creating an atmosphere up to 28% oxygen at some times.

Before being placed in the chamber, the animals were exercised to near exhaustion by swimming for 30 min. in water at 35 C. For Exp. 1, a glass aquarium with water 21 cm deep was used, but because the rats were able to prop themselves up on their tails for as much as a minute at a time and thus avoid swimming, a large vat with water 30 cm deep was used for Exp. 2. The rats were removed from the swim tank at the end of 30 min., momentarily wrapped in a towel to remove excess water and placed in the chamber of the respirometer. Within 5 min. after the animal had been lifted from the swim tank, the instrument was activated and oxygen consumption was being measured.

The first 300 cc oxygen was utilized in 15 to 20 min. and the piston was thus returned to an empty position. During the next 3 min. another 300 cc oxygen was pumped into the chamber, moving the piston to the "full" position. As the oxygen was used, the recording pen connected with the piston progressed across the chart on the instrument making a dip at one minute intervals (Fig. 1). The procedure was repeated for 90 min. at which time the animal had

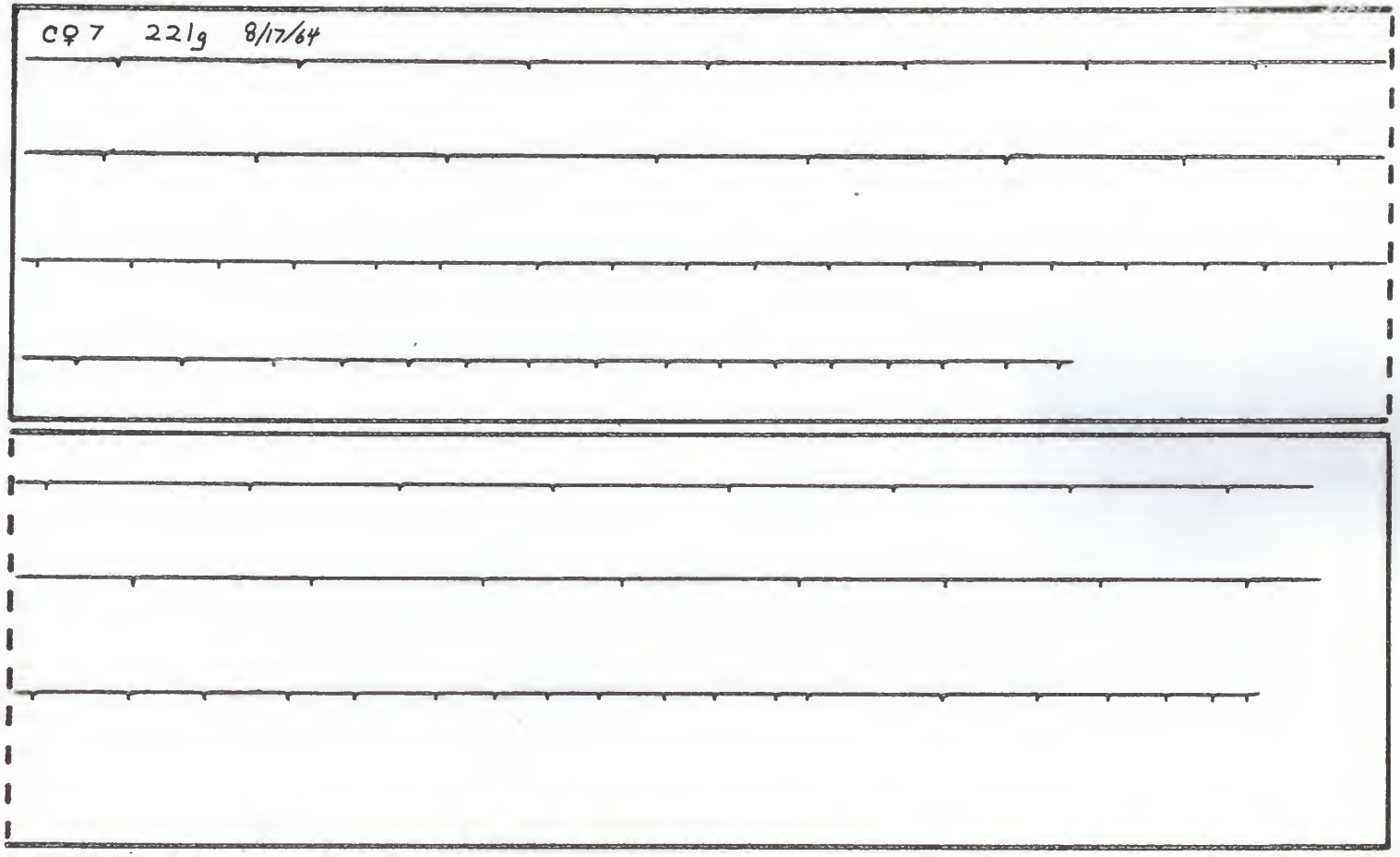


Fig. 1. Copy of chart of oxygen uptake following exercise as recorded by the "Minute Oxygen Uptake Spirometer" for one full run of approximately 90 min. The chart is reproduced as if the original had been cut in half, as the total chart was 20 in. long.

recovered from the increased oxygen uptake resulting from the swim. With the gradual decrease in uptake, each progressive line of the chart took a longer time than the one preceding it. The animal was returned to its cage at the completion of the experiment.

The charts were evaluated individually. The instrument was calibrated so that 1 mm of pen travel was equal to 2/3 cc volume reduction in the chamber. Sixty minutes was chosen as the time for comparison because after that time little additional recovery occurred and in some animals recovery was complete. The amount of oxygen used during refill was estimated by measuring the pen travel immediately before and after the 3 min. interval and interpolating the gap.

Three groups of mice were maintained on control, 0.01 and 0.001% octacosanol supplemented diets under the same conditions as described previously for the rat experiments. Five mice from a group were exercised and their oxygen uptake measured as described above. Animals of the same weights were selected from each group so that there was no weight differences between groups to be considered. The three groups were run once each week for seven consecutive weeks. The respirometer was set up as described for the rats in Exp. 2. Exercise of the mice was the same as for the rats and the recorded oxygen uptake was treated in the same manner.

RESULTS AND DISCUSSION

Conception and Reproduction, Maintenance and Growth of Young

Data were collected for a comparison of the conception rates of female rats on control feed (Table 2) versus those on experimental feed (Table 3). The numbers under the column headed first litter, first mating were obtained by counting the days from the first day that male and female were placed

Table 2. Breeding performance of female rats on control feed. Time was tabulated from the first day of cohabitation to parturition. If parturition did not occur within 35 days, a male was again introduced for "second mating." In the animal number, C = "control", E = "experimental", the digital number represents the generation, and the tens, the individual animal number.

Animal Number	First litter			Second litter		
	First mating	Second	Third	First	Second	Third
10-C	24			26		
20-C	32			25		
30-C	27			27		
40-C	34			24		
50-C	28			25		
60-C	25					97
70-C	--			--[1]		
80-C		58			62	
90-C		60			61	
100-C			97	25		
110-C	27			28		
120-C	24			24		
	average 39.6			38.5		
11-C	27			29		
21-C	27			25		
31-C	26			27		
41-C	27			28		
51-C		61		26		
61-C	26			26		
71-C	23			27		
81-C	31			27		
91-C	24			26		
101-C	24			25		
	average 29.6			26.6		
12-C	31					129[2]
22-C	25					142[2]
32-C	27				62	
42-C			129[2]	25		
52-C	35					166[3]
62-C	30					97
72-C	35				61	
82-C	25					94
92-C	30			--[4]		
102-C	25			27		
	average 39.2			89.2		

[1]Failed to produce a litter but necropsy revealed the presence of placental scars.

[2]Fourth mating. [3]Fifth mating. [4]Did not produce a second litter.

Table 3. Breeding performance of female rats on 0.1 percent octacosanol supplemented feed and calculated as described for Table 2.

Animal Number	First litter			Second litter		
	First mating	Second	Third	First	Second	Third
10-E	27			27		
20-E		62		27		
30-E			94	26		
40-E	30			25		
50-E	31			--[1]		
60-E	25			26		
70-E	30			37		
80-E	25			25		
90-E	24			28		
100-E	31			26		
110-E	25			25		
120-E		59		29		
130-E	26			26		
	average 37.6			27.3		
11-E	39			27		
21-E	25			28		
31-E	25				61	
41-E	26			26		
51-E	28			25		
61-E	32			30		
71-E	27			27		
81-E	27				59	
91-E	27			25		
101-E	30			31		
111-E	34			31		
121-E	28			24		
131-E	26				61	
141-E	25			33		
151-E	28			27		
161-E	25			30		
	average 28.3			34.1		
12-E	28			27		
22-E	37			26		
32-E	27				67	
42-E	23				60	
52-E	36					94
62-E	24					97
72-E	27				59	
82-E	33			25		
92-E	25				62	
102-E	25					98
112-E	25					128[2]

Table 3. (concl.)

Animal Number	First litter			Second litter		
	First mating	Second	Third	First	Second	Third
122-E	30			--[3]		
132-E	26			--[3]		
142-E	31			25		
152-E	33					97
162-E	27					93
	average 28.6			68.5		

[1]Died five days after parturition. [2]Fourth mating.
[3]Did not produce a second litter.

together to the day of parturition. The numbers under the column headed first litter, second (mating), were obtained by adding 35 days to the days from the first day of cohabitation to parturition as 15 days were allowed for mating and 20 days for parturition or indication of advanced pregnancy. Numbers under the column headed first litter, third mating, were the days from the first day of cohabitation to parturition plus 70 days. The numbers in the columns under the heading second litter were obtained in the same manner. In a few instances, fourth or even fifth matings were necessary in which cases 105 and 140 days were added to the days from the first day of cohabitation to parturition.

There was some variation evident among the groups. However, since control and experimental groups were under the same conditional influences, the results of the two treatments were comparable. The average times required for conception and parturition of each group as given in Tables 2 and 3 are shown graphically in Fig. 2.

In all cases but one, the average time required for conception and parturition by the octacosanol groups was less than that required by the control groups. The shortest time and the greatest number of days required, as

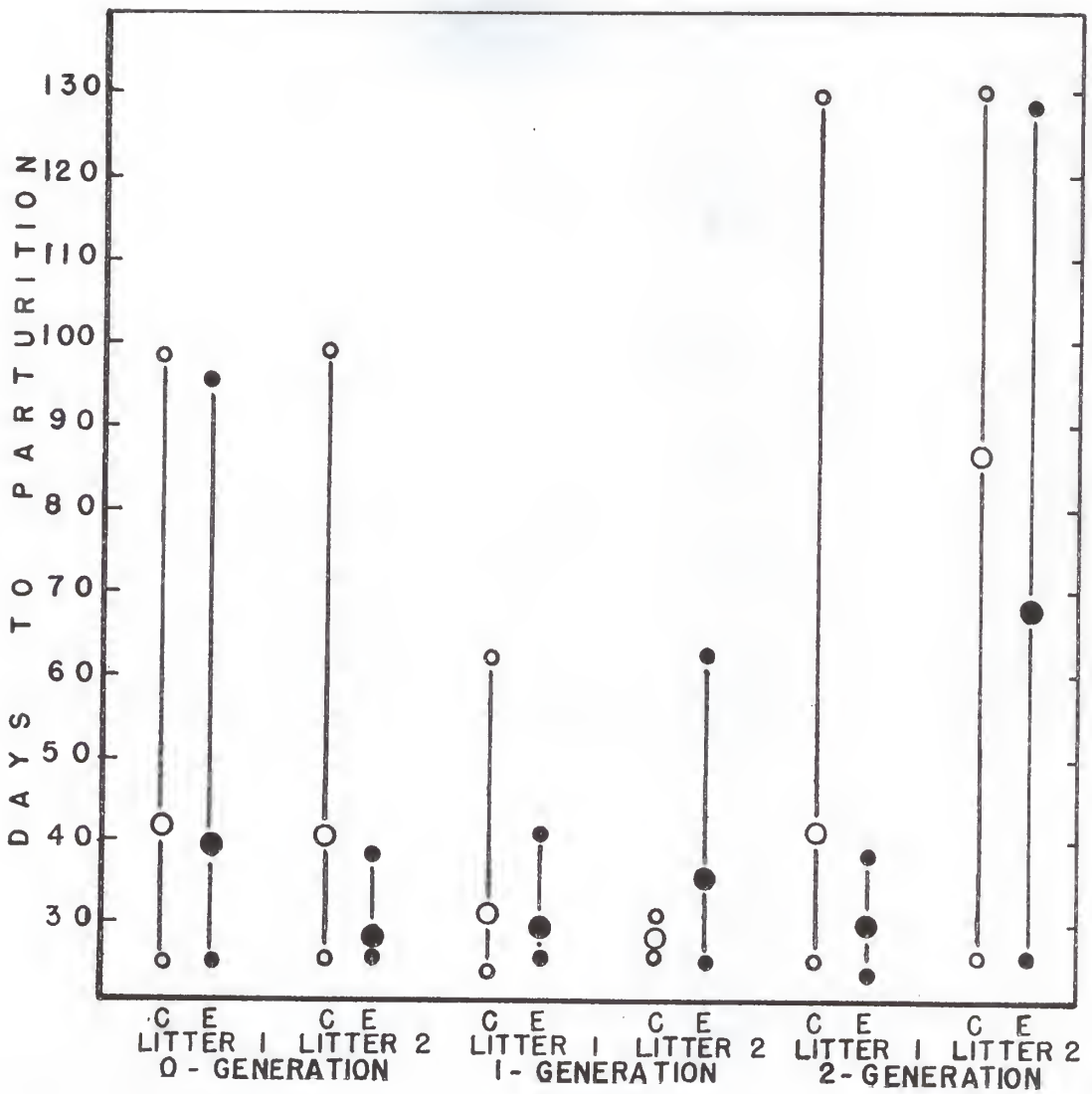


Fig. 2. Comparison of time from the first day of cohabitation to parturition by groups as listed in Tables 1 and 2. The large dots and circles represent the means and the small dots and circles the extremes of the time required for parturition by control and octacosanol groups respectively.

well as the averages, are designated in the graph. The average number of days for parturition within any group was shifted upward in many cases due to the requirement of a few females for several successive matings. The shortest breeding periods and the low breeding average indicated that most females conceived during the first period with the male.

The factor of wheat germ oil essential for reproduction found by Evans and Bishop (1923) and isolated by Evans and Burr (1924) from the unsaponifiable fraction of wheat germ oil was probably octacosanol. Wheat germ oil was demonstrated as an aid to conception in rats by Levin, Sibernagel, and Nichols (Neufeld, 1960) and in repeat-breeder cows (Marion, 1962). The observed results were considered by Evans and Burr (1924) to occur from prevention of resorption of embryos. It was difficult to account for the action of such a minute quantity of octacosanol as was present in wheat germ oil, or even the amount used in these tests. Increased conception and decreased resorption may result from better physical condition of the dam, slight increase in oxygen carrying capacity of the blood, or better utilization of oxygen or food. The only difference between the treatments was the presence of octacosanol in the experimental group so the difference in conception rate must be due to that substance, as previous results were considered to be due to wheat germ oil.

In all but one group in the present study, the rats on octacosanol reproduced, on an average, in less time than the controls. In generation 1, litter 2, all the control females conceived within the first 15 days breeding period while three of the experimental females required re-breeding. Discounting the disproportionately long times for conception in generation 2, litter 2, because of general health conditions in the colony,[2] the average time from

[2] In July 1964, the colony became seriously infested with mites, which caused considerable irritation, hair loss, and scaliness of skin. On August 8, all the animals in both the control and experimental groups were treated by

cohabitation to conception was 34.9 days in the control group and 31.1 days in the octacosanol group. This difference only borders on statistical significance because of the reversal of the trend in generation 1 litter 2. We believe the difference is real, and in general, a 10% reduction in breeding time can be expected with rats receiving octacosanol in their diet.

Litter size at birth was not greatly different between control and octacosanol groups (Tables 4 and 5), with an overall average litter of 9.5 in the controls and 9.2 in the octacosanol group. The difference was due primarily to less extremely large litters in the octacosanol groups, and a higher proportion of good healthy litters of 7 to 12 pups each in the control groups.

Reduction of large litters to a maximum of ten pups on day five actually reduced pup loss, and resulted in larger, healthier pups at weaning time. At weaning, on day 21, the octacosanol group had maintained slightly larger litters than the control animals, and although the difference was determined to be statistically nonsignificant, the trend was consistent. Average litter size was determined for both day one and day 21 by dividing the total number of pups by the number of litters on day one. Only three litters were lost completely out of 45 in the octacosanol groups and two out of 32 were lost from the control groups.

---

mist spray with a new arachnacide, "Vapona" or "DDVP" (0,0-dimethyl-0-2, 2-dichlorovinyl phosphate) produced by Shell Chemical Co. The treatment was highly effective for the mites, but every pregnant female resorbed her embryos, and the lactating females showed almost complete loss of milk, with resulting death of young pups and drastic loss of weight of the older pups. Only a few of the females in either group had weaned the first litter. All the litters that were lost were struck from the tabulations, and the females were retained for another mating. Complete recovery was not attained until early November. One of the controls and two of the experimentals never parturated before the project was terminated in January, 1965. In general, recovery was slightly more rapid in the animals with octacosanol in the diet.

Table 4. Size of litters and weight of pups from female rats on the control feed. All large litters were reduced to ten on day five.

Animal Number	First litter				Second litter			
	Number of young		Weight of young		Number of young		Weight of young	
	Day 1	Day 21	Total	Average	Day 1	Day 21	Total	Average
10-C	15	7	242	35	12	8	224	28
20-C	10	10	408	41	11	9	253	29
30-C	10	8	356	44	1	0	---	--
40-C	13	8	348	43	12	10	296	30
50-C	10	6	280	47	11	10	292	29
60-C	9	8	413	52	8	8	352	44
70-C	--	--	---	--	--	--	---	--[1]
80-C	10	4	229	57	10	10	565	57
90-C	11	9	276	31	14	10	541	54
100-C	11	10	427	43	12	10	438	44
110-C	12	10	403	40	11	10	385	39
120-C	9	8	368	46	13	10	446	45
average	10.9	8.0	341	42.6	10.4	8.6	379	39.9
11-C	4	4	220	55	11	4	184	46
21-C	11	10	414	41	9	4	202	51
31-C	12	10	423	42	15	10	377	38
41-C	12	10	420	42	11	10	420	42
51-C	4	4	242	61	10	9	405	45
61-C	14	10	464	46	14	10	415	42
71-C	15	10	368	37	12	10	401	40
81-C	7	2	110	55	9	6	281	47
91-C	5	0	---	--	11	8	367	46
101-C	7	7	242	35	9	8	312	39
average	9.1	6.7	323	43.3	11.1	7.9	336	42.6
12-C	7	6	226	38	11	10	427	43
22-C	9	9	181	20	1	1	45	45
32-C	5	4	124	31	7	7	322	46
42-C	5	5	198	40	3	3	138	46
52-C	11	9	247	27	6	4	195	49
62-C	9	9	233	26	3	3	135	45
72-C	8	7	283	40	9	8	377	47
82-C	12	10	260	26	12	10	414	41
92-C	14	10	323	32	--	--	---	--[2]
102-C	7	7	268	38	7	3	139	46
average	8.7	7.6	234	30.8	6.6	5.4	244	44.7
grand average	9.6	7.5	300	38.9	9.5	7.4	322	41.9

[1]Did not produce a litter. [2]Did not produce a second litter.

Table 5. Size of litters and weight of pups from female rats on the 0.1 percent octacosanol supplemented feed. Large litters reduced to ten on day five.

Animal	First litter				Second litter			
	Number of young		Weight of young		Number of young		Weight of young	
Number	Day 1	Day 21	Total	Average	Day 1	Day 21	Total	Average
10-E	11	10	396	40	10	10	304	30
20-E	14	10	310	31	9	9	415	46
30-E	7	7	353	50	8	7	366	52
40-E	13	8	352	44	12	10	304	30
50-E	12	0	---	--	--	--	---	--[1]
60-E	10	9	374	41	9	9	307	34
70-E	8	6	308	51	6	6	267	44
80-E	12	8	298	37	13	10	283	28
90-E	11	10	412	41	11	9	382	42
100-E	11	10	428	43	12	10	301	30
110-E	9	9	391	43	11	10	390	39
120-E	12	10	441	44	11	6	314	52
130-E	10	10	417	42	14	10	349	35
average	10.8	8.2	373	41.9	10.5	8.8	332	37.6
11-E	11	10	435	44	8	8	324	41
21-E	10	10	392	39	5	5	208	42
31-E	11	10	393	39	7	6	310	52
41-E	13	10	396	40	11	6	320	53
51-E	4	4	243	61	12	6	319	53
61-E	8	8	361	45	9	9	294	33
71-E	11	10	365	37	9	9	391	43
81-E	9	9	376	42	11	10	373	37
91-E	5	5	246	49	4	4	186	47
101-E	8	8	385	48	7	7	309	44
111-E	7	7	352	50	4	2	118	59
121-E	9	9	345	38	6	6	284	47
131-E	10	8	351	44	11	10	434	43
141-E	12	10	352	35	11	8	390	49
151-E	12	10	405	41	8	8	487	61
161-E	9	0	---	--	8	0	---	--
average	9.3	8.0	360	42.2	8.2	6.5	316	45.6
12-E	7	4	154	39	7	6	330	55
22-E	7	7	198	28	4	4	204	51
32-E	9	9	248	27	8	8	350	44
42-E	11	0	---	--	11	9	335	37
52-E	6	3	98	33	10	10	382	38
62-E	7	7	222	32	9	9	385	43
72-E	9	7	314	45	10	10	351	35
82-E	8	8	312	39	9	9	384	43
92-E	9	8	271	34	11	10	391	39
102-E	10	10	316	32	2	2	86	43

Table 5. (concl.)

Animal Number	First litter				Second litter			
	Number of young		Weight of young		Number of young		Weight of young	
	Day 1	Day 21	Total	Average	Day 1	Day 21	Total	Average
112-E	7	7	248	35	11	10	351	35
122-E	11	8	210	26	--	--	---	--[2]
132-E	9	8	239	30	--	--	---	--[2]
142-E	7	7	264	38	9	6	277	46
152-E	10	10	201	20	7	7	268	38
162-E	8	7	223	32	7	7	330	47
average	8.4	6.9	235	32.0	8.2	7.6	316	41.3
grand average	9.4	7.7	312	38.8	8.9	7.5	313	41.5

[1]Died. [2]Did not produce a second litter.

Litter weights varied with the size of the litter, and somewhat between generations, with the 2C-1 and 2E-1 litters drastically reduced in weight because of conditions in the colony.[2] There were no significant or consistent differences between octacosanol and control groups except as reflected by litter size. Individual weights varied inversely with litter size and only in the 0-2 litter was any other variation noticeable. In that case the controls were slightly larger even though the litter size was the same.

Levin, Sibernagel, and Nichols (Neufeld, 1963) demonstrated that wheat germ oil in the diet of rats favorably influenced litter size, birth weights and weaning weights. Keane (1953) found that wheat germ oil aided the maintenance of pups to weaning age. Growth of rats was aided by wheat germ oil (Blumberg, 1933). In the present work, octacosanol appeared to be an adjunct to maintenance of litters, but no influence on weaning weights could be detected.

Tabulated data on weights of intact animals and of individual organs (Tables 6,7,8,9) showed variation, particularly among the six groups. The variation may be explained partially by the age of the animals at necropsy.

Experimental and control females were sacrificed on day 21 after they had produced a second litter. Since considerable variation was evident in a comparison of the number of days from the first day of cohabitation to parturition, a variation in animal age and size would be expected. The parental generation animals were from 188-303 days old when sacrificed. Group "1" was from 170-242 and group "2" from 264-373 days old. The males were sacrificed after the second litters had been produced, a few being kept until all the females requiring more than one mating had parturated a second litter.

When adjustment was made for weight variation, the only difference between the treatments was the kidney weight of the females. The difference was statistically significant at the 1.0% level, with the experimental females having a slightly smaller kidney weight. Testis and testis plus epididymus weights were slightly greater in the octacosanol group, exactly in proportion to body weights.

The level of octacosanol used in this set of feeding trials was approximately equivalent to that which would be supplied in pure wheat germ oil, or 10 to 100 times that which would be given in normal feeding experiments or treatment regimens with wheat germ oil. In no instance was there any indication of reduced function or impaired structures in any of the test animals that could in any way be related to the octacosanol in the feed.

Oxygen Uptake

Two sets of experiments on oxygen uptake are tabulated in Tables 10 and 11. Statistical analysis showed no significant difference in the oxygen uptake of animals when adjustment was made for weight but in Exp. 1 there was a

Table 6. Total and organ weights of female rats on the control diet.

Animal number	Total weight	Kidneys	Liver	Spleen	Heart
10-C	226	2.0	7.8	0.4	0.9
20-C	210	2.2	7.4	0.5	0.9
30-C	310	2.3	9.8	0.8	1.1
40-C	255	2.3	8.8	0.5	1.1
50-C	210	2.0	7.5	0.4	0.8
60-C	320	2.2	14.5	0.7	1.3
70-C	310	2.4	10.0	1.0	1.4
80-C	280	2.5	13.0	0.9	1.1
90-C	286	2.4	12.0	0.9	1.1
100-C	284	3.2	13.1	0.7	1.0
110-C	272	2.1	10.5	0.8	1.0
120-C	255	2.4	11.0	0.8	1.2
average	268	2.3	10.5	0.7	1.1
11-C	310	3.2	14.5	1.3	1.5
21-C	250	2.2	11.5	0.5	0.9
31-C	213	1.1	7.2	0.5	0.8
41-C	237	2.1	12.5	0.8	1.0
51-C	238	1.9	11.6	0.4	0.9
61-C	233	2.1	11.3	0.8	1.4
71-C	231	1.8	10.8	0.8	1.3
81-C	233	2.0	8.6	0.4	0.9
91-C	260	1.9	14.1	0.7	0.9
101-C	222	2.1	8.0	0.8	1.4
average	243	2.0	11.0	0.7	1.1
12-C	292	2.3	16.1	0.6	1.2
22-C	285	2.2	10.2	0.7	1.1
32-C	274	2.1	16.6	0.5	1.0
42-C	275	2.2	11.8	0.6	1.1
52-C	270	1.7	11.7	0.6	0.8
62-C	257	1.8	10.1	0.6	1.0
72-C	257	2.0	10.8	0.5	0.9
82-C	270	2.0	11.5	0.4	1.1
92-C	290	2.0	11.3	0.8	1.0
102-C	251	1.5	10.2	0.4	1.0
average	272	2.0	12.0	0.6	1.0
grand average	261	2.1	11.1	0.7	1.1

Table 7. Total and organ weight of female rats on the 0.1 percent octacosanol supplemented diet.

Animal Number	Total weight	Kidneys	Liver	Spleen	Heart
10-E	226	2.0	8.6	0.6	1.0
20-E	330	2.2	17.0	0.5	1.2
30-E	340	2.8	15.0	1.0	0.9
40-E	232	2.0	8.8	0.5	0.9
50-E	---	---	----	---	---[1]
60-E	217	1.8	7.7	0.5	0.9
70-E	250	1.8	9.0	0.4	1.0
80-E	212	1.8	6.8	0.3	1.0
90-E	282	2.1	10.5	0.7	1.4
100-E	217	2.1	7.7	0.5	1.1
110-E	240	2.2	10.0	0.9	0.9
120-E	255	2.8	13.6	0.5	1.1
130-E	240	1.9	10.0	0.7	0.8
average	253	2.1	10.4	0.6	1.0
11-E	228	1.9	9.5	0.6	1.0
21-E	241	1.4	8.6	0.4	0.9
31-E	260	2.1	10.7	0.6	1.1
41-E	243	2.1	12.4	0.7	1.1
51-E	262	1.9	9.7	0.7	1.0
61-E	237	1.6	8.8	0.4	1.1
71-E	254	1.7	9.4	0.4	0.8
81-E	237	2.0	14.1	0.6	1.0
91-E	243	1.7	9.4	0.6	0.8
101-E	217	1.5	8.4	0.4	0.8
111-E	256	1.9	7.9	0.8	1.3
121-E	221	1.7	9.7	0.5	0.9
131-E	264	2.1	12.9	0.5	0.9
141-E	238	1.9	11.7	0.5	1.1
151-E	191	1.7	7.5	0.5	0.8
161-E	258	1.9	12.0	0.5	0.7
average	241	1.8	10.2	0.5	1.0
12-E	232	2.1	12.9	0.5	1.0
22-E	242	1.9	9.6	0.5	1.0
32-E	255	1.8	12.0	0.5	0.9
42-E	290	2.2	13.4	0.5	1.1
52-E	240	2.1	11.0	0.5	1.0
62-E	265	1.7	10.0	0.5	0.9
72-E	310	2.1	16.0	0.6	1.1
82-E	272	2.0	14.8	0.5	0.9
92-E	285	1.9	13.9	0.6	1.0
102-E	274	2.2	13.2	0.6	1.1
112-E	330	2.3	14.9	0.7	1.1
122-E	275	1.6	7.0	0.7	0.9

Table 7. (concl.)

Animal number	Total weight	Kidneys	Liver	Spleen	Heart
132-E	265	1.6	8.1	0.8	0.9
142-E	285	1.9	11.5	0.5	1.0
152-E	240	1.9	9.8	0.5	0.9
162-E	275	2.0	13.3	0.6	1.0
average	271	1.9	11.9	0.6	1.0
grand average	255	1.9	10.9	0.6	1.0

[1]Died after first parturition.

Table 8. Total and organ weights of male rats on the basic diet.

Animal number	Total weight	Kidneys	Liver	Spleen	Heart	Testis and epididymis	Testis
10-C	475	3.4	14.3	1.0	2.0	6.6	4.4
20-C	450	5.0	19.0	1.3	1.1	6.3	4.3
30-C	370	2.7	13.0	0.7	1.4	5.0	3.5
40-C	377	3.4	12.7	0.9	2.1	5.3	4.0
50-C	470	3.5	14.9	0.9	1.7	6.0	4.0
60-C	380	3.0	14.0	1.0	1.3	5.3	3.6
average	420	3.5	14.7	1.0	1.6	5.7	4.0
11-C	370	2.5	11.5	0.8	1.2	5.1	3.5
21-C	329	2.1	8.2	0.7	0.9	5.2	3.5
31-C	383	2.5	12.3	0.5	1.1	5.4	3.9
41-C	322	2.2	9.5	0.8	0.8	4.3	3.1
average	351	2.3	10.4	0.7	1.0	5.0	3.5
12-C	290	2.0	8.6	0.5	1.0	5.1	3.6
22-C	360	2.5	9.5	0.6	1.1	5.2	3.5
32-C	306	2.5	8.8	0.5	1.1	4.6	3.1
42-C	380	2.4	11.5	0.7	1.3	6.0	4.0
52-C	285	2.2	9.7	0.5	1.1	5.0	3.6
average	324	2.3	9.6	0.6	1.1	5.2	3.6
grand average	370	2.8	11.8	0.8	1.3	5.4	3.7

Table 9. Total and organ weight of male rats on the diet containing 0.1 percent octacosanol.

Animal number	Total weight	Kidneys	Liver	Spleen	Heart	Testis and epididymis	Testis
20-E	445	3.1	9.4	0.8	1.6	5.6	4.2
30-E	430	3.3	13.8	1.0	1.7	6.4	4.0
40-E	405	3.1	11.8	0.8	1.5	6.0	4.0
50-E	430	3.0	11.4	1.0	1.6	6.3	4.1
60-E	350	2.8	13.0	1.0	1.5	6.0	4.1
average	412	3.1	11.9	0.9	1.6	6.1	4.1
11-E	368	2.4	10.3	0.6	1.3	5.2	3.6
21-E	360	2.6	8.2	0.9	1.4	4.6	3.4
31-E	369	2.3	10.6	0.9	1.2	5.9	3.7
41-E	360	2.5	10.9	0.9	1.2	5.0	3.6
51-E	348	2.2	9.5	0.6	0.7	5.3	4.2
61-E	382	2.5	10.2	0.7	2.2	5.6	4.2
average	365	2.4	9.9	0.8	1.3	5.3	3.8
12-E	390	2.6	10.6	0.7	1.1	6.5	3.7
22-E	385	2.8	11.0	0.9	1.2	5.8	3.9
32-E	400	2.7	10.8	0.7	1.3	6.2	4.2
42-E	420	2.8	11.5	0.9	1.3	6.1	4.2
52-E	400	2.7	13.1	0.7	1.3	5.3	3.7
62-E	385	2.4	8.4	0.7	1.1	4.8	3.3
72-E	380	2.7	10.8	0.7	1.3	6.0	4.0
82-E	410	2.6	13.8	0.8	1.4	6.1	3.7
average	396	2.7	11.3	0.8	1.3	5.9	3.8
grand average	390	2.7	11.0	0.8	1.4	5.7	3.9

significant positive relationship between weight and oxygen uptake (Fig. 3). The line obtained by raising the body weight of the animals to the 3/4 power and relating that number to oxygen uptake fit the data well. A regression line showed 2.71 cc change in oxygen uptake during recovery for every gram change in weight.

For the animals on Exp. 2 the oxygen uptake was random irrespective of the weight. The rats on Exp. 1 were younger and lighter in weight than those on Exp. 2. More frequent runs for the animals on the second experiment may have resulted in some adaptation to swimming. The greater depth of water in the swim tank used in Exp. 2 must have increased the energy expended in exercise. Further, those animals on Exp. 2 were under the same unfavorable influences mentioned for the breeding colony.[2] Whatever the cause, many of the animals in Exp. 2 did not incur an oxygen debt relative to their body weight as did those in Exp. 1. In Exp. 2, when the average weights were adjusted for comparison, the debts for the 0.001 and the 0.01 groups were less than that for the controls of the same average weight.

No references were found on oxygen debt of rats under stress and on a diet supplemented with octacosanol. However, Ershoff and Levin (1955) and Consolazio et al. (1964) reported that octacosanol and wheat germ oil did not increase the time that rats could swim before drowning.

Wachholder (1948) reported that after exercise in the human there was a gradual decrease in oxygen uptake through a recovery period to a basal rate and fluctuations above and below the base line after the debt period. A similar pattern was exhibited by rats as can be visualized by plotting centimeters of pen travel of the respirometer recording device against time of recovery (Fig. 4). Both the oxygen debt and the fluctuations about a base line after recovery are evident.

Table 10. Oxygen utilization Exp. 1. Oxygen (cc) used during 60 min. recovery after 30 min. of exercise by rats of varied weights (g) and on diets of basic feed and four levels of octacosanol-supplemented feed.

Control		.0001		.001		.01		.1	
Weight	Oxygen	Weight	Oxygen	Weight	Oxygen	Weight	Oxygen	Weight	Oxygen
178	633	171	750	140	567	156	637	148	575
182	712	118	467	134*	529	211	715	174	620
178	655	154	523	141	563	152	563	155	531
146	547	177	675	182	653	111*	493	136*	549
164	657	184*	743	170	620	129*	523	182	675
192*	627	177	643	170	660	169	600	155	560
178	727	174	553	172	687	149	652	151*	740
188*	633	171	707	144	531	151	593	156	633
150	580	180*	747	161	598	153	627	166	624
184*	713	176	696			131*	530	163	645
179	655					144	523		
185*	707					141*	580		
average									
175	654	168	650	157	601	150	586	159	615
adjusted average*									
169	646	165	627	160	610	161	614	162	608

*These weights and oxygen measurements were eliminated to derive the adjusted average in which the animal weights among levels were less variable.

Table 11. Oxygen utilization Exp. 2. Oxygen used during 60 min. recovery after 30 min. exercise by rats on two levels of octacosanol-supplemented feed and control feed.

Control		.001		.01	
Weight	Oxygen	Weight	Oxygen	Weight	Oxygen
211	800	217	1000	211	830
223	870	204	755	207	855
229**	864	216	900	209	924
227**	965	217	953	215	998
220	985	211	1037	226	872
214	1046	211	720	241*	775
206	808	212	800	208	659
221	1070	199	853	217	767
196	826	185**	688	209	743
197	740	190	848	221	925
201	920	197	747	195	910
202	740	186**	943	203	1050
203	1100	182**	649	211	934
201	1000	188	690	207	937
189	987	196	730	207	1040
193	803			214	1048
202	970			233*	997
305*	1000			229	875
259**	780			202	897
300*	713			207	867
average					
220	899	201	821	214	895
adjusted average*					
211	904			211	896
adjusted average**					
205	911	205	836		

*These animals were eliminated to derive the same average weight for the control group and the 0.01 group.

**The animals marked * and ** were eliminated to get the same average weight for the control group and the 0.001 group.

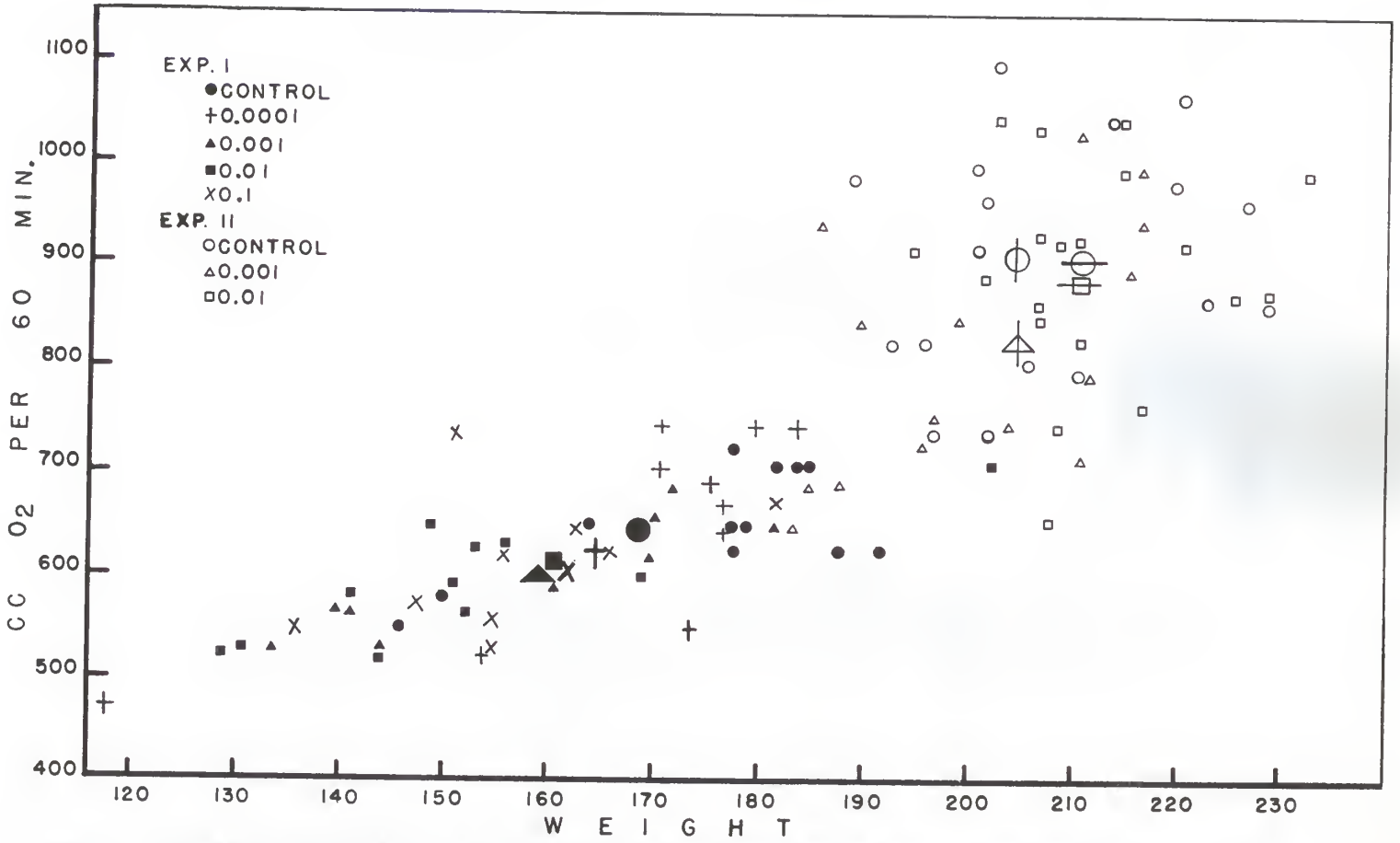


Fig. 3. Oxygen uptake during 60 min. recovery after exercise related to the weight of the rat. Symbols are explained in the legend. Exp. 1 rats demonstrated a relationship of oxygen used during recovery to the weight. Exp. 2 rats showed a random uptake of oxygen irrespective of weight.

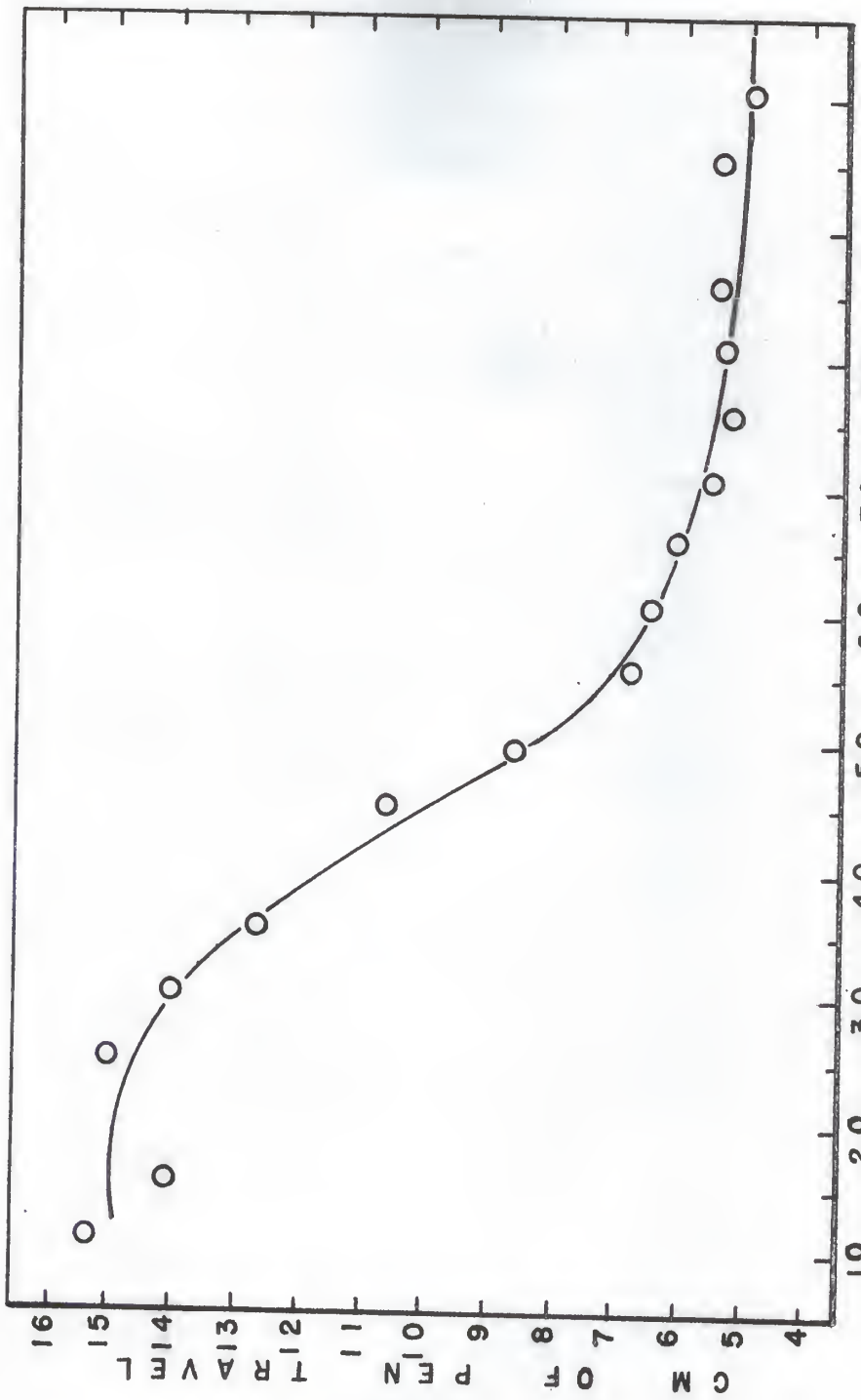


Fig. 4. Oxygen uptake following exercise as determined from the chart in Fig. 1. Each circle represents the amount of oxygen utilized in a 5 min. period.

The study with rat respiration points the way for a more clearly defined study to be undertaken in the near future in which the actual work performed by the rat will be measured. This should provide a better basis for comparison of the debt incurred by the individual animals.

When the three groups of five mice each were run on the respirometer for seven consecutive weeks statistically significant differences at the 0.01 level were obtained among the groups on control and two levels of experimental feed. In addition to the distinct difference among treatment groups a gradual adaptation to exercise was demonstrated by the week by week decrease in the oxygen used in 60 min. after exercise, even in the control group (Fig. 5). The amount of wheat germ oil, and octacosanol, as feed additives necessary to produce maximum physiological results has been shown by Keane (1953) and Neufeld (1963) to be quite low, with 1 or 2% wheat germ oil maximal for most reported successful work. Wheat germ oil contains 0.11% octacosanol, so the normal administration of 1% wheat germ oil would provide 0.0011% octacosanol. This is the level at which greatest reduction of oxygen debt occurred in both the rats and the mice subjected to swimming stress. The feeding experiments, with about 100 times the recommended levels of octacosanol, were conducted basically to determine if such levels of the substance would show any toxic effect. It is surprising that such massive dosage would lend any stimulatory effects on reproduction or on oxygen utilization.

Another factor that could have influenced the results was the ration that was used in the experiments. Previous reports of favorable effects of wheat germ oil and octacosanol usually resulted from addition of wheat germ oil to a highly purified, and possibly inadequate diet. The ration used in the present series of experiments was a complete feed, with adequate or excess of all known complements. Addition of 0.0001% octacosanol to such a complete

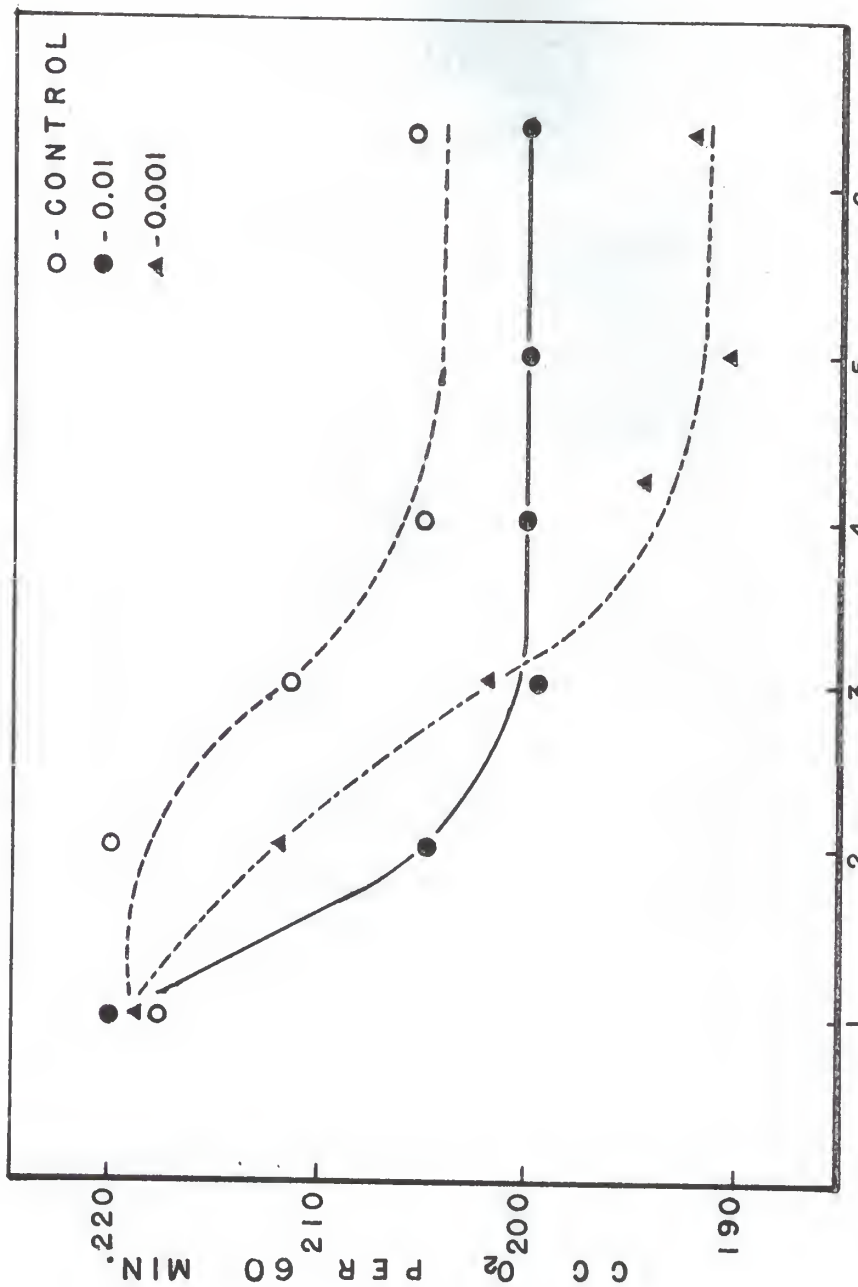


Fig. 5. Oxygen uptake by mice following exercise. Symbols are explained in the legend. Mice receiving the octacosanol supplemented diet used less oxygen during 60 min. recovery than did the mice on the control diet. Each group of mice used less oxygen during recovery with progressive weeks of exercise.

feed should, according to all known principles of nutrition, have produced no effects.

In the present series, the animals receiving octacosanol at the 0.01 or 0.001% levels as food additives generally excelled those without the additive. Animals receiving 0.1% octacosanol equalled or slightly exceeded the performance of the animals on equivalent feed without octacosanol. Results from this study thus support, although not conclusively, that octacosanol may be one of the active ingredients of wheat germ oil.

SUMMARY

Little information was available on the effects of octacosanol on rats and none on its effects on oxygen uptake. A series of experiments was devised to determine some of those effects. Two sets of Sprague Dawley rats were maintained for the study, one on a complete ration and the other on the same ration with octacosanol added. Comparisons were made of time required for reproduction, size of litters, weight of young, weight of adult rats and weight of selected organs. In another series, oxygen utilization by rats on control feed and on various levels of octacosanol was determined after exercise.

Experimental rats required slightly less time for reproduction than did those on control feed (31.1 vs. 34.9 days) reflecting higher conception rates in the experimental animals. Octacosanol may function as a preventive against resorption by promoting the health of the dams, or aiding oxygen or food utilization.

Litter size at birth did not differ between the two sets of animals. By day 21 the octacosanol groups had maintained more litter members. Litter weights varied directly and individual pup weights inversely with the size of

the litter. No difference in weight was detected between octacosanol and control groups. There was some indication that octacosanol may function in litter maintenance.

Weights of the adult animal and weights of selected organs were subject to variation due to the age of the animals at necropsy. When organ weights were corrected for body weight no significant difference was found between the treatments for the males, but the kidneys of the experimental females were significantly lighter than those of the controls.

Determination of oxygen uptake after 30 min. swimming showed considerable variation within treatment groups. A direct relation between the oxygen used during recovery and the weight of the animal was evident in rats of 130 to 185 g. No such relationship was found in animals over 200 g probably due to the method of swimming and adaptation. Adult rats that received 0.01 and 0.001% octacosanol in the feed required consistently less oxygen during the recovery period than animals of the same weight on control feed. The weight to the 3/4 power is directly proportional to the oxygen needed for 60 min. recovery after comparable exercise. The increment was determined as 2.71 cc per gram body weight.

The recovery pattern of mice was similar to that of rats. The mice receiving the octacosanol supplement (0.01 and 0.001%) required less oxygen for recovery than the controls (statistically significant at the 0.01 level). Gradual adjustment to exercise was evident since the uptake decreased with each consecutive week of the experiment, in all groups.

Octacosanol, as a food additive, provided results equal or superior to those obtained from comparable animals maintained on the same food without octacosanol.

ACKNOWLEDGMENTS

Acknowledgment is gratefully made to Dr. H. T. Gier for his understanding guidance during the time of this study and in the preparation of this thesis.

Acknowledgment is also due the Department of Zoology and the Department of Dairy Science for laboratory space and for research equipment. Financial support for the study was provided by Dr. E. Levin, VioBin Laboratories, Monticello, Illinois.

The author would also like to express an appreciation to professors and to fellow students for their suggestions and assistance in fulfilling the requirements for this degree.

LITERATURE CITED

Asdell, S. A. 1949. Nutrition and the treatment of sterility in dairy cattle: Review. J. Dairy Sci., 32:60-70.

Blumberg, H. 1935. A growth deficiency, curable by wheat germ oil. J. Biol. Chem. 108(1):227-238.

Bramante, P. O. 1959. Apparatus for automatically recording oxygen consumption, respiratory movements and activity of small animals. J. Appl. Pnysiol. 14:1063.

Bramante, P. O. 1961. Quantitation of oxygen consumption and spontaneous muscular activity of the rat. J. Appl. Physiol. 16(6):982-990.

Card, L. E. 1929. Vitamin E requirements of poultry. Poult. Sci. 8(6): 328-334.

Carver, D. S. and Johnson, R. L. 1953. Unidentified growth factors for the chick in vegetable oils and fatty acid concentrates. Poult. Sci. 32(4): 701-705.

Consolazio, F. C., L. O. Matoush, R. A. Nelson, G. J. Isaac and L. M. Hursh. 1964. Effects of octacosanol, WGO, and vitamin E on performance of swimming rats. J. Appl. Physiol. 19(2):265-267.

Cromer, J. K. 1938. Wheat germ oil in treatment of repeated spontaneous and threatened abortion. Med. Ann. Dist. Col. 7:145-148. (Nutrition Abstr. 8:481).

Cureton, T. K. 1954. Effect of WGO and vitamin E on normal human subjects. Am. J. Physiol. 179:628.

Cureton, T. K., and R. H. Pohndorf. 1955. Influence of WGO as a dietary supplement in a program of conditioning exercises with middle-aged subjects. Research Quarterly. 26:391-407.

Davis, J. E. 1938. The effect of advancing age on the oxygen consumption of rats. Am. J. Physiol. 119:28.

Davis, J. E., and A. B. Hasting. 1934. The measurement of the oxygen consumption of immature rats. Am. J. Physiol. 109:683-687.

Emerson, G. A. and H. M. Evans. 1937. The effect of vitamin E deficiency upon growth. J. Nutrition. 14(2):169-178.

Ershoff, B. H. and E. Levin. 1955. Beneficial effect of an unidentified factor in wheat germ oil on the swimming performance of guinea pigs. Fed. Proc. 14:431-432.

Evans, J. M. and K. S. Bishop. 1923. Existence of a hitherto unknown dietary factor essential for reproduction. J. Am. Med. Ass'n. 81(11):889-892.

Evans, H. M. and K. S. Bishop. 1924. Stability and solubilities of the food substances or vitamine X required for reproduction. Anat. Record. 27(4): 203-204.

Evans, H. M. and G. O. Burr. 1924. Preliminary steps in the isolation and concentration of vitamine X. Anat. Record. 27(4):203.

Evans, H. M. and G. O. Burr. 1928. Development of paralysis in the suckling young of mothers deprived of vitamin E. J. Biol. Chem. 76(1): 273-297.

Goettsch, M. and J. Ritzman. 1939. The preventive effect of wheat germ oils and of alpha-tocopherol in nutritional muscular dystrophy of young rats. J. Nutrition. 17(3):371-381.

Gopinath, K. W., J. M. Kohli and A. R. Kidwai. 1962. Chemical examination of Sarcococca pruniformis: identification of the neutral constituents. J. Sci. Ind. Rech. 21B:459. (Chem. Abstr. 58:2476c).

Gullickson, T. W., L. S. Palmer, W. L. Boyd, J. W. Nelson, F. C. Olson, C. E. Calverley and P. D. Boyer. 1949. Vitamin E in the nutrition of cattle: I. Effects of feeding vitamin E poor rations on reproduction, health, milk production, and growth. J. Dairy Sci. 32:495.

Gwatkin, R. and A. H. MacLeod. 1938. Wheat germ oil in Brucella infection in cattle and guinea pigs. Canad. J. Comp. Med. 2(5):133-136.

Holtkamp, D. E., S. Ochs, C. C. Pfeiffer and A. E. Heming. 1955. Determination of oxygen consumption of groups of rats. Endocrinology 56:93.

Keane, K. W. 1953. Reproductive failure of rats on glyceryl trilaurate-containing diets and its prevention by certain natural fats. (Dissertation Publ. 5231) (Biol. Abstr. 28:8056).

Kleiber, M., A. H. Smith and T. N. Chernikoff. 1956. Metabolic rate of female rats as a function of age and body size. Am. J. Physiol. 186:9.

Lentz, R. W. 1938. Correction of sterility in cows with wheat germ oil preparation enoulan. Gerlin tierarstl Wochenschr. No. 14:201-202. (Nutrition Abstr. 8:360).

Levin, E. 1945. Vitamin E vs. wheat germ oil. Amer. Jour. Digest. Diseases. 12(1):20-21.

Levin, E. 1963. Effects of octacosanol on chick comb growth. Proc. Soc. Exptl. Biol. Med. 112:331-334. (Chem. Abstr. 59:4238f).

Levin, E., W. Silbernagel and F. A. Nichols. 1950. Unidentified factors in wheat germ oil influencing reproduction. (A preliminary report) Denison Univ. J. Sci. Lab. 49(5):6-11.

MacLagan, N. F. and M. M. Sheahan. 1950. The measurement of oxygen consumption in small animals by a closed circuit method. J. Endocrinol. 6:456.

Marion, G. B. 1962. Effects of wheat germ oil on reproductive efficiency in repeat-breeder cows. J. Dairy Sci. 45(7):904-906.

Martin, G. J. 1937. Vitamin E. J. Nutrition. 13(6):679-685.

Milhorat, A. T., V. Toscani and W. E. Bartels. 1945. Effect of wheat germ on creatinuria in dermatomyositis and progressive muscular dystrophy. Proc. Soc. Exp. Biol. Med. 58:40.

Neufeld, G. J. 1963. Estrogenic-androgenic effects of wheat germ oil and octacosanol. Master's thesis, Kansas State University.

Pacini, A. J. 1938. The "saturation" method for the treatment of breeding failures with wheat germ oil. J. Am. Vet. Med. Ass'n. 83(4):236-238.

Sadykov, A. S., I. Kh. Isaw and A. I. Ismailov. 1963. Isolation by extraction and separation of some substances from the cotton plant. Uzbeksk. Khim. Zh. 7(2):53-56. (Chem. Abstr. 59:10473).

Salisbury, C. W. 1944. A controlled experiment in feeding wheat germ oil as a supplement to the normal ration of bulls used for artificial insemination. J. Dairy Sci. 27(7):551-562.

Stone, S. 1949. The therapeutic and prophylactic administration of wheat germ oil (vitamin E) in infancy and childhood. Arch. Pediatrics. 66(5):189-200.

Sure, B. 1924a. Dietary requirements for reproduction. II. The existence of a specific vitamin for reproduction. J. Biol. Chem. 58(3):693-709.

Sure, B. 1924b. Dietary requirements for reproduction. III. The existence of the reproductive dietary complex (vitamin E) in the ethereal extracts of yellow corn, wheat embryo, and hemp seed. J. Biol. Chem. 62(2): 371-396.

Sure, B. 1926a. Dietary requirements for reproduction. V. The role of various vegetable and fruit oils in fertility and lactation. J. Biol. Chem. 69(1):29-40.

Sure, B. 1926b. Dietary requirements for reproduction. VII. The existence of a lactation-promoting factor in the unsaponifiable matter from wheat oil. J. Biol. Chem. 69(1):53-74.

Sure, B. 1927. Dietary requirements for reproduction. XI. The potency of butter fat in vitamin E. J. Biol. Chem. 74(1):71-84.

Titus, H. H. and W. H. Burrows. 1940. Influence of wheat germ oil on semen production of cockerels. Poult. Sci. 19(5):295-298.

Tracy, P. H., W. A. Hoskisson and J. M. Trimble. 1944. Wheat germ oil as an antioxidant in dairy products. J. Dairy Sci. 27(4):311-318.

Tuma, J. W. 1959. The influence of exercise and diet supplement wheat germ oil on fitness changes during exercise. Doctorate's Dissertation, Univ. of Illinois. (Dissertation Abstr. XX-3189).

von Noorden, C. Metabolism and practical medicine. Vol. 1. Chicago: W. T. Keener and Co., 1907.

Wachholder, K. 1948. Energiebedarf für körperliche Arbeit und Erhaltungsumsatz Pflugers Arch. Physiol. 250(4):534-551. (Biol. Abstr. 23:20886).

Watson, E. N. and W. P. Tew. 1936. Wheat germ oil therapy in obstetrics. Am. J. Obstet. Gynecol. 31:352-357. (Nutrition Abstr. 6:183).

White, A., P. Handler, E. L. Smith and D. Stetten. 1959. Principles of biochemistry. 2nd. ed. New York: McGraw-Hill Book Co., Inc.

Zollner, N. ed. 1962. Thannhauser's textbook of metabolism and metabolic disorders. Vol. 1. New York: Grune and Stratton.

THE EFFECTS OF OCTACOSANOL ON CONCEPTION AND REPRODUCTION, ON MAINTENANCE AND GROWTH OF YOUNG, AND ON OXYGEN UPTAKE IN THE WHITE RAT

by

PATRICIA ROSE FARRELL

B. S., Mount Saint Scholastica College, 1963

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1966

Wheat germ and wheat germ oil have been noted as beneficial dietary supplements by playing a role in earlier conception, maintenance of pregnancy, and increased stamina. Octacosanol is a long chain alcohol which is isolated from wheat germ oil and is responsible for some of the effects of wheat germ oil.

The purpose of this study was to test the octacosanol derivative of wheat germ oil as a causative agent for some of the effects attributed to wheat germ and wheat germ oil and to determine if excessive amount of octacosanol produce adverse effects. Rats were chosen as the experimental animals and handled according to the procedure prescribed by the Pure Food and Drug Administration. Three generations of animals were maintained on control and on 0.1% level of octacosanol-supplemented diets. Records were kept of the number of days from the first day of cohabitation to the day of parturition, of the number in litters on days 1 and 21, of the weight of litters, and of the weight of the adult animals and selected organs of the adult animals. Measurements of oxygen uptake after exercise were made on rats and mice of various weights and of 0.1, 0.01, 0.001, and 0.0001% levels of octacosanol-supplemented feed.

Experimental rats required slightly less time for reproduction than did those on control feed (31.1 vs. 34.9 days), reflecting faster conception rates in the experimental animals. Octacosanol may function as a preventive against resorption, by promoting the health of the dams, or by aiding oxygen or food utilization.

Litter size at birth did not differ between the two sets of rats. By day 21 the octacosanol groups, had, with the exception of one group, maintained more members of the litters. Litter weights varied directly and average pup weights inversely with the size of the litter. No difference in weight was

detected between octacosanol and control groups. There was some indication that octacosanol may function in litter maintenance.

Weights of the adult rat and weights of selected organs were subject to variation due to the age of the rats at necropsy. When organ weights were corrected for body weight no significant difference was found between the treatments for the males, but the kidneys of the experimental females were significantly lighter than those of the controls.

Determination of oxygen uptake after 30 min. swimming showed considerable variation within treatment groups. A direct relation between the oxygen used during recovery and the weight of the animal was evident in rats of 130 to 185 g. No such relationship was found in rats over 200 g probably due to the method of swimming, the factor of adaptation and bouyancy of the older and heavier animals. The weight to the 3/4 power is directly proportional to the oxygen needed for recovery after comparable exercise. The increment was determined as 2.71 cc per gram body weight. Adult rats that received 0.01 and 0.001% octacosanol in the feed required consistently less oxygen during the recovery period than animals of the same average weight on control feed.

The recovery pattern of mice was similar to that of rats. The mice receiving the octacosanol supplement (0.01 and 0.001%) required less oxygen for recovery than the controls (statistically significant at the 0.01 level). Gradual adjustment to exercise was evident since the uptake decreased with each consecutive week of the experiment, in all groups.

Octacosanol, as a food additive, provided results equal or superior to those obtained from animals maintained on the same food without octacosanol.